REMARKS

Claim 4-7, 10, 14-16, 20, 22, 23, 28, 29, 35, 43-65, and 69-74 are withdrawn; claims 3, 17, 21, 26, 27, 30, and 31 are amended; and claims 1-3, 8, 9, 11-13, 16-19, 21, 24-27, 30-34, 36-42, and 66-68 are pending. Claims 17-19 and 21-27 are rejected under 35 U.S.C. § 112, second paragraph; claims 1-3, 8, 9, 11-13, 17-19, 21, 24-27, 30-34, 36-42, and 66-68 are rejected under 35 U.S.C. § 112, first paragraph. Each of the rejections is addressed below.

Support for the Amendments

Support for the amendments is found throughout the specification and claims as originally filed. For example, support for the amendment of claim 3, which now recites administering an ezrin modulating agent "at or near a site where modulation is desired" is found at page 38, lines 8-10, and at page 26, lines 1 and 2. Other amendments to the specification and claims were made merely to correct typographical errors or to bring the specification and claims into compliance with U.S. patent practice. No new matter has been added.

Amendment and cancellation of the claims here are not to be construed as an acquiescence to any of the rejections/objections made in the instant Office Action or in any previous Office Action, and were done solely to expedite prosecution of the application. Applicants hereby reserve the right to pursue the claims as originally filed, or substantially similar claims in one or more subsequent patent applications.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 17-19 and 21-27 are rejected under 35 U.S.C. 112, second paragraph, for alleged indefiniteness. Applicants respectfully disagree and traverse the rejection. However, without acquiescing in any way to the rejection and in order to expedite prosecution and facilitate allowance of the application, Applicants have amended the claims. Accordingly, the indefiniteness rejection should be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-3, 8, 9, 11-13, 17-19, 21, 24-27, 30-34, 36-42, and 66-68 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. The rejected claims are directed to methods for modulating endothelial cell (EC) proliferation in a mammal by increasing or decreasing ezrin activity (claims 1-3, 8, 9, 11-13, and 66-68); methods for inducing formation of new blood vessels in a mammal by decreasing ezrin activity (claims 17-19, 21, 24-27, and 66-68); or methods for reducing the severity of blood vessel damage in a mammal by decreasing ezrin activity (claims 30-34, 36-42, and 66-68). In support of the enablement rejection, the Examiner asserts that Applicants have allegedly failed to provide *in vivo* data. In particular, at page 7, line 22, to page 8, line 3, the Examiner states:

> There are no examples of in vivo data in the specification, which provides only *in vitro* experimental data. Biological systems are complex, and while cells in an *in vitro* system may behave in a certain way, it is not necessarily predictive of *in vivo* activity.

For the reasons detailed below, Applicants respectfully disagree with the enablement rejection and request that it be withdrawn.

1. *In vivo* increase in endothelial cell proliferation results from reducing ezrin activity

Applicants disclose that disruption of ezrin activity *in vivo* increased endothelial cell proliferation and angiogenesis in a mouse hind limb ischemia model as shown in Applicants' specification at Example 14 (pages 45-46) and at Figure 13. In this example, Applicants injected mice with hindlimb ischemia with human umbilical vein endothelial cells (HUVEC) transfected with wild-type ezrin or with an ezrin protein having a dominant-negative mutation. Applicants discovered that mice that received HUVEC transfected with the mutant form of ezrin had significantly higher numbers of proliferating endothelial cells than control mice that received HUVEC transfected with wild-type ezrin. These *in vivo* results corroborate Applicants' *in vitro* data, which was presented at Example 12 (pages 43-44), where Applicants showed that tumor necrosis factor (TNF) treatment substantially reduced the number of proliferating endothelial cells, but that cells transfected with an ezrin protein containing a dominant negative mutation exhibited

significantly increased levels of proliferation. These results confirm that increasing or decreasing ezrin activity is sufficient to modulate endothelial cell (EC) proliferation as recited in claim 1, from which claims 3, 8, 9, 11-13, and 66-68 depend.

2. *In vivo* increase in blood vessel formation results from reducing ezrin activity

Applicants also provide *in vivo* data showing that decreasing ezrin activity increased blood vessel formation (page 12, lines 1-4, Figure 13, and Example 14, page 45, line 13, to page 46, line 21). Hindlimb ischemia was induced in mice by excision of the femoral artery. The ischemic hindlimb tissue was injected with HUVEC transfected with wild-type ezrin or with ezrin having a dominant negative mutation that reduced ezrin activity. Mice that received HUVEC having reduced ezrin activity showed a functionally significant increase in blood vessel formation as evidenced by a marked increase in hindlimb perfusion. No such increase in perfusion was observed in mice that received HUVEC transfected with wild-type ezrin. These results clearly demonstrate that decreasing ezrin activity increases blood vessel formation as recited in claim 17, from which claims 18, 19, 21, 24-27, and 66-68 depend.

3. Blocking RhoA kinase activity relieves ezrin/TNF mediated inhibition of endothelial cell proliferation

Following angioplasty, local expression of TNF at sites of arterial injury reduces endothelial cell proliferation and re-endothelialization of the damaged blood vessel (page 39, line 20, to page 40, line 6). These negative changes can be blocked by decreasing ezrin activity as evidenced in Examples 12 and 13, where Applicants show that decreasing ezrin activity blocks TNF's suppression of endothelial cell proliferation. In addition, Applicants have shown that blocking RhoA kinase activity using Y27632 reversed ezrin/TNF mediated inhibition of endothelial cell proliferation (Figure 15C and Example 16, page 47, line 25, to page 48, lines 1-11). Based on these results, one skilled in the art would expect that decreasing ezrin activity should reduce the severity of blood vessel damage when a mammal is exposed to conditions conducive to damaging the blood

vessels as acknowledged by the Office at page 6 of the Office action mailed August 1, 2006, where the Office states:

> Example 16 demonstrates that *in vitro*, treatment with Y27632 reversed ezrin/TNF mediated inhibition of endothelial cell proliferation in a dose dependent manner. **Such results would indicate that Y27632 could be useful *in vivo* for treating ischemic vascular disease** by enhancing endothelial cell. [*sic*] (Emphasis added.)

In sum, Applicants have demonstrated that decreasing ezrin activity should reduce the severity of blood vessel damage when a mammal is exposed to conditions conducive to damaging the blood vessels as recited in claim 30, from which claims 31-34, 36-42, and 66-68 depend.

The standard for enablement set forth in 35 U.S.C. 112, first paragraph, requires that Applicants provide a description of the invention sufficient "to enable any person skilled in the art to which it pertains . . .to make and use" the invention. Applicants' specification clearly describes methods that would enable one skilled in the art to modulate endothelial cell (EC) proliferation, increase angiogenesis, and reduce the severity of blood vessel damage by increasing or decreasing ezrin activity. In particular, Applicants have provided *in vivo* and *in vitro* working examples showing that increasing or decreasing ezrin activity is sufficient to modulate endothelial cell proliferation, to increase angiogenesis in a mouse model of hindlimb ischemia, and to reduce the severity of blood vessel damage in a mammal exposed to conditions conducive to damaging the blood vessels. The Office must provide specific technical reasons showing why one skilled in the art could not practice the invention without undue experimentation. M.P.E.P. 2164.04 In the absence of such evidence or reasoning, the enablement rejection should be withdrawn.

In support of the enablement rejection the Office has cited Uchida et al., (Biochemical and Biophysical Research Communications 269:633-640, 2000; hereinafter "Uchida"); Shibata et al., (Circulation 103:284-289, 2001; hereinafter "Shibata"); and Xue et al., (Hepatology 499, 38 (4) Suppl 1: 400A; hereinafter "Xue"). Regarding these references, the Office states, "However, the teachings of the art teach away from the claimed invention." The Office's reliance on these references is misplaced because the question of whether or not a reference "teaches away" from Applicants' claimed method is irrelevant to the question of enablement. *Singh v. Brake*, 317 F.3d 1334 (Fed. Circ. 2002). The Federal Circuit states:

> Although the questions (1) whether or not a reference "teaches away" from a claimed invention and (2) whether or not a claimed invention provides "unexpected results are relevant in determining whether or not a claimed invention would have been obvious, **they are not the primary questions bearing on enablement**. *Singh v. Brake*, 317 F.3d 1334, 1346 (Fed. Circ. 2002) (Citations deleted; emphasis added.)

The proper test of enablement is whether Applicants' specification teaches one of skill in the art to "make and use" the claimed invention. As detailed above, Applicants have plainly satisfied this standard. Accordingly, the enablement rejection of the claims should be withdrawn.

CONCLUSION

In view of the above amendment, Applicants believe the pending application is in condition for allowance.

Applicants believe that no additional fee is due to consider the present amendment. Nevertheless, the Director is hereby authorized to charge or credit any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105.

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Respectfully submitted,

By _____

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